Toxicity and kinetic studies of H^+, K^+-ATPase sensitivity to cyfluthrin and fenpropathrin in Egyptian field populations of *Spodoptera littoralis*

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ABSTRACT

Toxicity of two pyrethroids (cyfluthrin and fenpropathrin) against cotton leafworm, *Spodoptera littoralis*, larvae were collected from heavily sprayed fields in different areas in Dakahlia and El-Beheira Governorate. H^+, K^+-ATPase (proton-pump) activity was measured in laboratory (lab.) strain and compared with enzyme activities for other collected strains. The sensitivity of H^+, K^+-ATPase activity was measured by I_{50} values. The I_{50} values of cyfluthrin were 0.24; 0.46; 0.54; 0.65, and 0.68 µM for lab; Abo-Hommos; Shobrakheet; Aga, and El-Senbellaween strains respectively, while I_{50} values of fenpropathrin were 0.44; 0.58; 0.66; 0.84, and 0.87 µM for lab and four field strains respectively. Also, proton-pump enzyme kinetic parameters, K_m (Michaelis-Menten kinetics, constant) and V_{max} (maximum velocity) values and the inhibition constant (K_i) were determined. The obtained data proved that cyfluthrin and fenpropathrin compounds were competitive inhibitors of proton-pump activity. Results indicated that, the pyrethroid have shown potentiality against larvae of *S. littoralis,* so, these pyrethroids may be effect of a novel mechanism of action for inhibition of proton-pump activity, these recommended for *S. littoralis* larvae control. It could be concluded that the use of pyrethroid, may avoid increasing of resistance by effect of new mode of action where they disrupt the development of target pest, Therefor proton-pump may play an important role the mode of action of these pesticides in future.

Key words: H+, K+-ATPase, Protein-pump, cyfluthrin- Fenpropathrin.

INTRODUCTION

As a basis for developing strategies to delay or avoid insecticide resistance to commercial chemical agents, it is important to understand the physiological defensive system that might be brought to bear when a pest species is challenged be exposure to different classes of insecticides (Bull, 1992). Factors that contribute to the development of insecticide resistance have been extensively investigated in a large number of species (Oppenorth, 1985). The mechanism of resistance is complex and sometimes varies in the different insect biotypes to the same insecticide.

The extensive use of insecticides to control of *Spodoptera littoralis* larval instars, the larvae has led to the development to multiple insecticide resistance, the control achieved is not successful because of the insect's high capacity to develop resistance toward the majority of these compounds (Temerak, 2002). Therefore, there is a great need to develop novel alternative control agents with new mode of action where they disrupt the development of target pest, or functional combinations of pest control techniques is emphatically a product of this decade to reduce resistance to conventional insecticides, the pyrethroides have proved a powerful insecticide control tool which has in some cases threatened their longer term viability through rapid development of pest resistance (Dent, 2000).

In this work, we measured the activity and sensitivity of H^+, K^+-ATPase (proton-pump) to cyfluthrin and fenpropathrin. We also, studied the enzyme kinetic for the H^+, K^+-ATPase in four field strains and compared them with data obtained of laboratory strain.

MATERIALS AND METHODS

1. Tested insects:

Susceptible laboratory strain of cotton leafworm, *Spodoptera littoralis* was provided by Central Lab. of Pesticides, Agricultural Research Center (ARC) Cairo, Egypt which was reared for several years on artificial diet under standard laboratory conditions of 27 ± 2 ^oC and 65-70 % RH.

Field strains of cotton leafworm, *Spodoptera littoralis* egg masses were collected from cotton fields at Abo-Hommos and Shobrakheet (El-Beheira Governorate); Aga and El-Senbellaween (Dakahlia Governorate); the 4th larval instar used for toxicity and kinetic studies.

2. Tested insecticides:

Cyfluthrin (baythroid, 5% EC), was obtained from Bayer AG, Germany, and fenpropathrin (meothrin 20% EC)), was obtained from Sumitomo, Japan.

3. Bioassay tests:

3.1. Toxicity of the tested pyrethroids against S. littoralis:

Cyfluthrin and fenpropathrin were bioassayed against the 4th larvae of *S. littoralis*. The castor leaves were dipped in different concentrations of the two pyrethroid. cyfluthrin and fenpropathrin concentrations were prepared in distilled water. Treated and control leaves plants were air-dried for 3 hr, the treated leaves were placed in clean glass container at the laboratory conditions of (27 ± 2 °C) and 65-70 % RH, ten larvae (lab and four field strains) were used for each test with three replicate at least. Number of alive and dead larvae per replicate was counted 24, and 48 hr, after treatment. Concentrations-mortality percentage were calculated and corrected for natural death according to Abbott equation (Abbott, 1925). LC_{50} values were calculated and statisticaly and analysed by using the probit-analysis method of Finney (1971).

4. Biochemical studies:

4.1. Brush Border Membrane Vesicles (BBMV) preparation:

Brush Border Membrane Vesicles (BBMV) was prepared from *S. littoralis* 4th instar larvae (lab and four field strains). Larvae were isolated mid-guts, the mid-guts larvae were weighted and placed in a vail with nine times their weight of ice cold buffer A (300 mM mannitol, 5 mM EGTA and 17 mM Tris-HCl pH 7.4) (Biber *et al*., 1981). Mid-guts were homogenized in buffer A, an equal volume of 24 mM $MgCl_2$ solution was added to the mid-guts homogenate, then centrifuged at 4500 r.p.m for 15 min at 4 °C, the supernatant centrifuged at 16.000 r.p.m for 30 min at 4 °C. The pellet was resuspended in 0.5 ml homogenate volume of ice cold buffer A and centrifuged at 4500 r.p.m for 15 min at 4 °C, the pellet was resuspended in 0.5 homogenate volume of ice cold buffer A and recentrifuged at 16.000 r.p.m for 30 min at 4 °C. The pellet constituted the BBMV preparation according to the method of Wolfersberger *et al*., (1987). All the centrifugation preparation were accomplished by Beckman J2-21 rotor.

4.2. Assay of BBMV proton-pump (H^+, K^+-ATP) activity:

The proton-pump (H^+, K^+-ATP) activity measurements was done according to the method reported by Yoda and Hokin (1970), in total volume of 1 ml, ten µl of BBMV as a source of proton-pump was added to the reaction mixture (500 µl) containing 20 mM Kl_2, 5 mM $MgCl_2$, 40 mM Tris-HCl buffer (pH 7.4), to this mixture 5 mM adenosine-5-triphosphate disodium salt (ATP) was added, the volume was then directly adjusted to 850 µl by 40 mM tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 250 mM Sucrose (TES), the mixture was incubated for 15 min in a shaking water bath at 37 °C, the reaction was stopped by adding 150 µl Trichloroacetic acid (TCA), 50 %. The inorganic phosphate (Pi) liberated from the hydrolysis of ATP by enzyme was determined colorrimetrically (Model 340 Spectrophotometer) by adding 4 ml color reagent remained of ferrous sulfate (5 %), ammonium molybdate (10 %) in sulfuric acid (10 N), according to the method described by Taussky and Shorr (1953), the intensity of the developed colour was measured spectrophotometrically at λ 740 nm. The enzyme specific activity was presented as 25 µmol Pi/mg protein/hr.

The protein content of the homogenates of *S. littoralis* was determined by the method of Lowry *et al*. (1951) at λ750 nm using Bovine Serum Albumin (BSA) as a standard protein.

4.2. *In vivo* inhibition of proton-pump (H^+, K^+-ATP) activity:

The inhibition percentage of proton-pump activity was determined in the 4th instar larvae

previously feed on leaves treated with the concentration of LC_{50} values of each of the tested insecticides (cyfluthrin and fenpropathrin). 10 µl of the enzyme preparation was incubated with the substrate for 30 min, the enzyme-substrate mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{V - V_i}{V} \times 100$$

Where:-

(V) is the specific activity in larvae feed on treated castor leaves.

(Vi) is the specific activity in larvae feed on non treated castor leaves.

4.2. *In vitro* inhibition of proton-pump (H^+, K^+-ATP) activity:

The inhibitor of proton-pump activity was evaluated to determine enzyme kinetic parameters, the method of Dixon and Webb (1964) was adopted to draw the Dixon-plots by plotting 1/V versus concentrations of the inhibitor (cyfluthrin and fenpropathrin) at two concentrations of the substrate, ATP (the substrate of ATPase) concentrations of 3.0 and 5.0 mM. Estimation of I_{50} value was carried out by preincubating the enzyme with the inhibitor for 30 min, using the following concentrations 0.1; 1; 5; 10; 50, and 100 µM. K_i (the inhibition constant) values for each inhibitor were estimated from Dixon-plot. K_m (Michaelis-Menten kinetics, constant) and V_{max} (maximum velocity) values were calculated by a linear regression of 6 point on each Lineweaver and Burk Plot (1934).

RESULTS AND DISCUSSION

Toxicity of two pyrethroid against *S. littoralis* larvae:

Toxicity of the two pyrethroid expressed in terms of LC_{50} are given in Table (1). Cyfluthrin LC_{50} values after 24 hr are 0.82; 1.19; 1.35; 2.58, and 2.97 ppm for lab; Abo-Hommos; Shobrakheet; Aga, and El-Senbellaween strains respectively, while LC_{50} values after 48 hr are 0.057; 0.35; 0.64; 0.67, and 0.89 ppm respectively. Also, fenpropathrin LC_{50} values after 24 hr are 0.36; 2.10; 2.44; 3.67, and 3.85 ppm for lab and four field strains respectively, while LC_{50} values after 48 hr are 0.098; 1.21; 1.44; 2.02, and 2.32 ppm respectively. According to LC_{50} values it is quite clear that the toxicity was higher with cyfluthrin and fenpropathrin for El-Beheira, while toxicity was low for Dakahlia, also, cyfluthrin was more toxic than fenpropathrin in controlling of *S. littoralis* larvae. The present results emphasize that during many years of selection pressure in the field, the resistance and/or tolerance levels to the conventional insecticides due to the intensive application of such for controlling *S. littoralis* larvae. The present results are confirmed by the results of investigators Kaygisiz, 1980; Bramhankar *et al*., 1990, and Temerak, 2002.

Table (1): Toxicity of cyfluthrin and fenpropathrin on *S. littoralis* larvae.

S. littoralis Strains	LC_{50} (ppm)			
	Cyfluthrin		Fenpropathrin	
	24hr	48hr	24hr	48hr
Lab	0.82	0.057	0.36	0.098
Abo-Hommos	1.19	0.35	2.10	1.21
Shobrakheet	1.35	0.46	2.44	1.44
Aga	2.58	0.67	3.67	2.02
El-Senbellaween	2.97	0.89	3.85	2.32

I*n vivo* inhibition of *S. littoralis* H^+, K^+-ATP activity:

The *in vivo* inhibitory effect of the LC_{50} values of tested pyrethroid against the *S. littoralis* 4th instar lab and four field strains larval proton-pump are shown in Table (2). The data cleared that cyfluthrin exhibited the high percentages of reduction of proton-pump activity, the percentages of proton-pump inhibition were 86.6; 72.4; 70.1; 64.3, and 60.0 % for lab; Abo-Hommos; Shobrakheet; Aga, and El-Senbellaween strains respectively. Also, in case of fenpropathrin percentages of proton-pump inhibition were 74.3; 63.4; 61.1; 54.2, and 53.1 %

for lab and four field strains respectively. It is clear that, the cyfluthrin and fenpropathrin active as inhibitor on proton-pump activity. These results are in agreement with investigators, Desaiag *et al*., 1975; Nelson, 1992, and Dean *et al*., 1999.

Table (2): *In vivo* inhibition of *S. littoralis* larvae proton-pump activity by two pyrethroids (LC_{50}).

S. littoralis Strains	% inhibition	
	Cyfluthrin	Fenpropathrin
Lab	86.6	74.3
Abo-Hommos	72.4	63.4
Shobrakheet	70.1	61.1
Aga	64.3	54.2
El-Senbellaween	60.0	53.1

Kinetic parameters of proton-pump inhibition:

The kinetic studies were conducted to evaluate the effects of cyfluthrin and fenpropathrin on proton-pump activity in both tested strains of *S. littoralis* 4th larvae, Table (3) shows the obtained Lineweaver-Burk (L-B) plots for proton-pump in lab and four field strains and the statistical analysis of the obtained values of K_m (Michaelis-Menten kinetics, constant) and V_{max} (maximum velocity) of the proton-pump. The K_m values for proton-pump were generally higher in all four tested field strains than lab strain, the change in K_m values of proton-pump between the four field strains indicated changes in the affinities.

The present results show that the V_{max} values of proton-pump was obviously higher, this points of the higher substrate turnover which may reflect the physiological importance of the proton-pump in the function of the nervous tissue of the *S. littoralis* larvae. The V_{max} values were generally higher in all four field strains than lab strain, this indicated that the number of active sites on the proton-pump of the larvae was increased in the field strains, such change may be followed by decrease in the insect susceptibility which could be altered by field application of the insecticides.

Table (3): Michaelies-Menten kinetics of the proton-pump of larval of *S. littoralis*, of cyfluthrin and fenpropathrin.

S. littoralis Strains	Cyfluthrin		Fenpropathrin	
	K_m mM	V_{max} mM	K_m mM	V_{max} mM
Lab	0.24	2.2	0.33	3.8
Abo-Hommos	0.39	3.6	0.54	4.8
Shobrakheet	0.53	4.5	0.60	5.3
Aga	0.67	6.3	0.77	6.9
El-Senbellaween	0.71	7.0	0.82	7.7

I*n vitro* inhibition of *S. littoralis* proton-pump activity:

To characterize more details about the *in vitro* inhibition of proton-pump by the inhibitors, the K_i value of each inhibitor was estimated from the graphical method of Dixon and Webb (1964), Table (4). The sensitivity of proton-pump activity to cyfluthrin and fenpropathrin were measured by I_{50} values. In the case of cyfluthrin the I_{50} values for were 0.24; 0.46; 0.54; 0.65, and 0.68 µM for lab; Abo-Hommos; Shobrakheet Aga, and El-Senbellaween strains respectively. Similarly, in case of the fenpropathrin the I_{50} values were 0.44; 0.58; 0.66; 0.84, and 0.87 µM for lab and four field strains respectively. The K_i values were 24; 43; 50; 65, and 73 µM for lab and four field strains

respectively, in case of cyfluthrin. Also, in case of fenpropathrin the values were 33; 57; 66; 79, and 85 µM for lab and four field strains respectively,

In comparing the inhibitory potency of cyfluthrin and fenpropathrin against proton-pump activity, it was clear that cyfluthrin and fenpropathrin showed to be the strong inhibitor for *S. littoralis* proton-pump activity, thus causing a decrease in the unidirectional transport of Na^+ and K^+ through cell membranes, these results suggest that proton-pump has major role in *S. littoralis* succeptibility to the two tested pyrethroid. These finding the confirmed by the results of authors such as, Dow, 1986; Wieckzoreck *et al*., 1989; Wieczorek, 1992; Christeller *et al*., 1992; Giordana and Parenti, 1994, and Dean *et al*., 1999.

Table (4): I*n vitro* inhibition of *S. littoralis* larvae proton-pump activity by two pyrethroid.

***S. littoralis* Strains**	**Cyfluthrin**		**Fenpropathrin**	
	I_{50} µM/L/min	**K_i µM**	**I_{50} µM/L/min**	**K_i µM**
Lab	0.24	24	0.44	33
Abo-Hommos	0.46	43	0.58	57
Shobrakheet	0.54	50	0.66	66
Aga	0.65	65	0.84	79
El-Senbellaween	0.68	73	0.87	85

In general, it may be suggest that proton-pump (H^+, K^+-ATP) could be used in the integrated pest management (IPM) programs, in order to maximize the effect of conventional insecticides when applied against *S. littoralis.*

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تأثير سمية وحركة التفاعلات ومدى حساسية إنزيم H+, K+-ATPase لمبيدي السيفلوثرين والفنبروباثرين في العشائر الحقلية المصرية لدودة ورق القطن (سبدوبترا ليتولارس)

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تم تقدير سمية أثنين من المبيدات البيرثرويدية سيفلوثرين وفنبروباثرين على العمر اليرقى الرابع لدودة ورق القطن بين أربع عشائر مختلفة جمعت من الحقول المصرية وتمت مقارنتها بعشيرة معملية، تركزت الدراسة على العشائر المنتشرة فى المناطق التى تم رشها بمعدل كثيف من المبيدات. وتم دراسة تأثيرالمركبين على نشاط أنزيم H^+, K^+-ATPase والذى يطلق عليه مضخة البروتون proton-pump للأربع سلالات حقلية تحت الدراسة. وأوضحت النتائج أن سلالات محافظة البحيرة كانت أكثر حساسية من سلالات محافظة الدقهلية للمركبين. وقد تم دراسة المقدرة التثبيطية للمركبين على نشاط أنزيم H^+, K^+-ATPase

وبعض الثوابت الخاصة بأنزيم H^+, K^+-ATPase مثل ثابت ميخائيل (K_m) وأقصى نشاط نوعى (V_{max}) وقيم الـ I_{50} للمعاملة *in vitro* كانت 0.24 ;0.46 ;0.54 ;0.65 و 0.68 ميكروموار بالنسبة لتأثير السيفلوثرين للسلالة المعملية، أبو حمص، شبراخيت، أجا و السنبلاوين على التوالى، أما بالنسبة للفنبروباثرين فكانت 0.58 ;0.66 ;0.84 0.44; و 0.87 للسلالة المعملية والأربع سلالات الحقلية تحت الدراسة على التوالى، وأيضاً تم تقدير قيم ثابت التثبيط K_i وقد أظهر المركبين تثبيط تنافسى على نشاط أنزيم H^+, K^+-ATPase. ومن هذه النتائج وجد أنه يمكن أستخدام هذا الأنزيم كهدف لفعل المبيدات البيرثرويدية فى مكافحة دودة ورق القطن وبذلك نتفادى زيادة مقاومة دودة ورق القطن لفعل المبيدات التقليدية.